PATENT ABSTRACTS OF JAPAN

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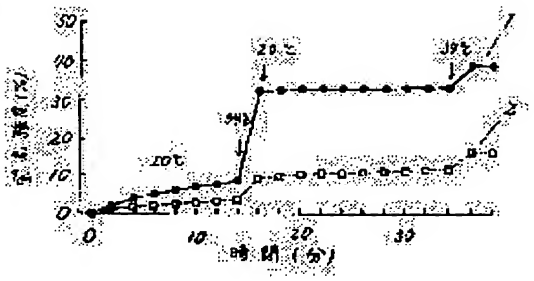
(54) TEMPERATURE SENSITIVE LIPOSOME

(57)Abstract:
PURPOSE: To obtain the temp. sensitive liposome which can release included materials at a specific temp. and is applicable in medical and pharmaceutical fields, such as drug carriers in drug delivery systems.
CONSTITUTION: A high-polymer compd. having clouding points is deposited on or in the conventional lipid liposome to obtain the temp. sensitive liposome. The high-polymer compd. deposited on or in the liposome is hydrophobed at the temp. near the clouding point of such liposome and, therefore, disturbance is generated in the structure of the liposome film and the release of the included materials arises. The high-polymer compd. having the clouding points is adequately polyacrylic acid polymers, such as poly-N-isopropyl acrylamide. The high- polymer compd. is stably deposited on the liposome surface if a hydrophobic group, such as alkyl group or cholesterol, is introduced therein.



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CLAIMS

[Claim(s)]
[Claim 1] It is temperature sensitivity liposome which ****** makes the interior support the high molecular compound which has a cloudy point, and changes.
[Claim 2] Temperature sensitivity liposome according to claim 1 to which the high molecular compound which has a cloudy point is characterized by being a polyacrylic acid system polymer.
[Claim 3] It is temperature sensitivity liposome which the interior is made to support ****** and changes by introducing a hydrophobic radical into the high molecular compound which has a cloudy point.
[Claim 4] Temperature sensitivity liposome according to claim 3 to which the high molecular compound which has a cloudy point is characterized by being a polyacrylic acid system polymer.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the temperature sensitivity liposome which can be applied in medicine and the pharmaceutical-sciences field and which may emit the endocyst matter corresponding to a temperature change including drugs and the charge of makeup.
[0002]
[Description of the Prior Art] Liposome is broadly applied as support, cell membrane models, etc., such as a drug, in medicine and the pharmaceutical-sciences field. Recently, liposome is macromolecule-ized, membranous is strengthened, or sugar, an enzyme, protein, etc. are fixed, stimulus responsibility liposome is prepared, and use is considered by the purpose of an absorptivity improvement of the drug to a living body as a drug carrier in a drug delivery system.
[0003] The application value of temperature sensitivity liposome making an endocyst drug emit in response to a living body's temperature, skin temperature, etc. is [in / medically and pharmacologically / the field of the charge of makeup] high among the above-mentioned stimulus responsibility liposome. Conventionally, as this temperature sensitivity liposome, dimyristoyl phosphatidyl choline, dipalmitoylphosphatidylcholine, JISUTE aroyl phosphatidylcholine, etc. had many things using the phospholipid which has a phase transition point. In phase transition temperature, a lipid changes from the gel state to a liquid crystal condition, turbulence arises in membrane structure and these emit the endocyst matter to it.
[0004] Moreover, liposome is covered with the polysaccharide derivative which introduced the Pluronic polymer which has a cloudy point near the room temperature as a part which picks up temperature, and the example which prepared the temperature sensitivity polymer is reported (the collection of the Society of Polymer Science, Japan drafts, 3020 1989 [-3022 or]).
[0005]
[Problem(s) to be Solved by the Invention] However, in the temperature sensitivity liposome prepared by conventional phospholipid, a setup near temperature was difficult so that the emission temperature of the endocyst matter may serve as a specific value by the lipid which constitutes the liposome film and it may apply in the living body. Moreover, emission of the endocyst matter was also slow and it was what it is hard to use to emit the drug of a considerable amount for a short time. In addition, emission might not take place almost according to the description of the matter which carries out endocyst etc.
[0006] Moreover, the liposome covered with the Pluronic polymer installation polysaccharide derivative was also unsuitable for the target site in the drugs which should be made to act for a short time, although the amount of said polymer introduced on liposome came out only, and did not react for whether being Sumiya to the temperature change for a certain reason but was fit for application to a sustained release drug. Furthermore, control of the amount of installation of the Pluronic polymer to a polysaccharide derivative was also difficult, and it was what cannot perform control of the responsibility or the burst size to a temperature change, and an emission rate easily.
[0007]
[Means for Solving the Problem] In order to solve the above-mentioned technical problem, when

the interior was made to support directly the high molecular compound which has a cloudy point and this high molecular compound carried out hydrophobing at the temperature more than a cloudy point, ****** of liposome changed the membrane structure of liposome, examines the system to which the endocyst matter is made to emit, and came to complete this invention.
[0008] Introduce hydrophobic radicals, such as an alkyl group and cholesterol, into the high molecular compound which is made to carry out the endocyst of the high molecular compound which has a cloudy point to the aqueous phase in liposome, or has a cloudy point, the interaction of this hydrophobic radical and the hydrophobic section of the liposome film is made to produce, and ****** of liposome makes the interior support said high molecular compound in this invention.
[0009] As a high molecular compound which has the cloudy point used by this invention, a polyacrylic acid system polymer can set up a cloudy point near temperature, and is especially desirable. In order to give a cloudy point to a polyacrylic acid system polymer, a straight chain, branched chain, or an annular hydrocarbon group is introduced into the carboxylic acid in a polymer chain, an amide, etc., and a polymer molecule is embellished. For example, the polymerization of the monomers, such as N-pentyl acrylamide, N-isopropyl acrylamide, and N-cyclohexyl acrylamide, can be carried out, and the suitable polymer for the purpose of this invention can be obtained. Furthermore, in case a polyacrylic acid system polymer is prepared, the cloudy point of a high molecular compound can be adjusted by carrying out copolymerization of the monomer from which versatility differs.
[0010] Moreover, if a hydrophobic radical is introduced into a high molecular compound in case especially a liposome front face is made to support said high molecular compound, it can be made to hold to stability. As a hydrophobic radical to introduce, long-chain ****** has a branching alkyl group, desirable cholesterol, etc.
[0011] In case the liposome used by this invention is prepared, there is especially no limit about the lipid to be used. In order to make liposome support a high molecular compound, it adds at the temperature below a cloudy point, the incubation of the solution of a high molecular compound is carried out to a liposome solution for a while, it carries out an interaction to it, or it mixes with a lipid at the time of liposome preparation, and endocyst is carried out into a liposome inland water phase.
[0012]
[Function] In the temperature sensitivity liposome which supported the high molecular compound concerning this invention, at the temperature below a cloudy point, if it becomes the temperature more than a cloudy point, the supported high molecular compound serves as hydrophobicity, and although it has spread underwater in the hydrophilic property, it tends to shift into a liposome lipid membrane at the same time a macromolecule chain contracts. Consequently, turbulence arises in the structure of a liposome lipid membrane, and emission of the endocyst matter takes place.
[0013] As described above, in emission of the endocyst matter reacted to the phase transition temperature of a lipid, it may almost be changeless depending on the matter to connote. However, in the case of the temperature sensitivity liposome concerning this invention, when the high molecular compound supported with the phase transition of a lipid serves as hydrophobicity, the burst size of the endocyst matter can be increased remarkably.
[0014] In case the liposome to which the endocyst of the drugs was carried out is applied in the living body, or the liposome to which the endocyst of the active principle was carried out is applied on the skin and the endocyst matter is made to emit in a target site, it is necessary to control an emission rate by an operation of drugs or the target site in various phases. In the temperature sensitivity liposome concerning this invention, the amount of the high molecular compound which liposome is made to support is easily controllable by changing the quantitative ratio of the high molecular compound which carries out an interaction to liposome. Therefore, it is possible to adjust extent of a structural change of the liposome lipid membrane to the temperature more than a cloudy point, and to control the burst size or emission rate of the endocyst matter.
[0015]

[Example] Furthermore, an example explains this invention to a detail.
[0016] As an example 1, the yolk lecithin multilamellar liposome which made Polly N-isopropyl acrylamide support was prepared. First, copolymerization of N-isopropyl acrylamide and the acrylic-acid stearyl was carried out by the mole ratio of 99:1, the copolymer (average molecular weight 20,000) was compounded, this copolymer water solution and the yolk lecithin multilamellar liposome solution prepared according to the conventional method were incubated at 20 degrees C for 12 hours, and the liposome front face was made to support Polly N-isopropyl acrylamide. Moreover, the carboxy fluorescein was used as endocyst matter.
[0017] About this Polly N-isopropyl acrylamide support liposome, the emission behavior of a carboxy fluorescein with time which carried out endocyst was investigated by measuring fluorescence intensity. The yolk lecithin liposome which is not making Polly N-isopropyl acrylamide support was made into the example 1 of a comparison at that time. A result is shown in drawing 1 .
[0018] In drawing 1 , the liposome of an example 1 showed the emission behavior of a carboxy fluorescein comparable as the liposome of the example 1 of a comparison at 20 degrees C, and the emission rate was controlled considerably. However, when temperature was raised at 34 degrees C, emission of a remarkable carboxy fluorescein was accepted in the liposome of an example 1. This emission was controlled when temperature was again reduced at 20 degrees C. On the other hand, the remarkable reaction to such temperature was not accepted in the liposome of the example 1 of a comparison. The remarkable temperature sensitivity in an example 1 is considered that the Polly N-isopropyl acrylamide which this liposome was made to support is because it has a cloudy point at 31 degrees C.
[0019] Next, the dipalmitoylphosphatidylcholine uni-lamellae liposome which made Polly N-isopropyl acrylamide support was prepared as an example 2. First, copolymerization of N-isopropyl acrylamide and the acrylic-acid stearyl was carried out by the mole ratio of 99:1, the copolymer (mean molecular weight 20,000) was compounded, this copolymer water solution and the dipalmitoylphosphatidylcholine uni-lamellae liposome solution prepared according to the conventional method were incubated at 20 degrees C for 12 hours, and the liposome front face was made to support Polly N-isopropyl acrylamide. Moreover, calcein was used as endocyst matter.
[0020] About this Polly N-isopropyl acrylamide support liposome, change by the temperature of the emission behavior of calcein which carried out endocyst was investigated by measuring fluorescence intensity. The dipalmitoylphosphatidylcholine liposome to which Polly N-isopropyl acrylamide is not carried out at the time of ** was made into the example 2 of a comparison at that time. The rate (leakage rate) to the intensive matter of the burst size of the calcein 1 minute [in each temperature] after measurement initiation showed the result to drawing 2 .
[0021] When the endocyst of the calcein is carried out, in usual lipid liposome, most change of emission by the phase transition temperature of a lipid is not accepted. Also in drawing 2 , although the phase transition temperature of dipalmitoylphosphatidylcholine was 42 degrees C, by the liposome of the example 2 of a comparison, the leakage of calcein was only accepted more slightly [in an elevated-temperature side] than near 42 degree C. On the other hand, in the liposome of an example 2, emission of calcein was promoted from near 31 degree C which is the cloudy point of Polly N-isopropyl acrylamide, and remarkable emission was further accepted near 42 degree C for a short time. In the liposome of an example 2, since the phase transition temperature of the dipalmitoylphosphatidylcholine from which the cloudy point of Polly N-isopropyl acrylamide constitutes 31 degrees C and liposome is 42 degrees C, it is thought that increase of emission of the endocyst matter is accepted in the large temperature requirement near [near 30 degree C to] 42 degree C. Moreover, even endocyst matter which is not usually emitted was able to make the considerable amount emit.
[0022]
[Effect of the Invention] As mentioned above, the temperature sensitivity liposome concerning this invention had the very good reactivity over specific temperature. Rather than the phase transition temperature of a liposome configuration lipid, since this good temperature sensitivity becomes settled by the cloudy point which the high molecular compound made to support has, it

can also set the temperature to which the endocyst matter is made to emit as desired temperature by adjusting the cloudy point of a high molecular compound.
[0023] Moreover, it was able to be made to emit good in the temperature sensitivity liposome concerning this invention also about the matter which was not able to cause emission sufficient in conventional temperature sensitivity liposome. It is possible to also make emission of a lot of endocyst matter cause for a short time from before by the synergism of change of the membrane structure by the phase transition of a liposome configuration lipid and change of the membrane structure by hydrophobing in the cloudy point of a high molecular compound. Therefore, in conventional temperature sensitivity liposome, when emission of drugs sufficient in a target site is not able to be achieved, there is application value.
[0024] Furthermore, in this invention, since the amount of support in the liposome of a high molecular compound which has a cloudy point is easily controllable, the emission rate of the endocyst matter from temperature sensitivity liposome is also controllable. Therefore, even when it applies to a drug delivery system etc., the difference of an operation of a target site and drugs, effectiveness, etc. can perform a dosage form design with a sustained release drug to the broad pharmaceutical preparation of immediate effect nature.
[0025] Thus, in medicine and the pharmaceutical-sciences field, useful temperature sensitivity liposome was able to be especially offered by this invention.

[Translation done.]

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TECHNICAL FIELD

[Industrial Application] This invention relates to the temperature sensitivity liposome which can be applied in medicine and the pharmaceutical-sciences field and which may emit the endocyst matter corresponding to a temperature change including drugs and the charge of makeup.

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PRIOR ART

[Description of the Prior Art] Liposome is broadly applied as support, cell membrane models, etc., such as a drug, in medicine and the pharmaceutical-sciences field. Recently, liposome is macromolecule-ized, membranous is strengthened, or sugar, an enzyme, protein, etc. are fixed, stimulus responsibility liposome is prepared, and use is considered by the purpose of an absorptivity improvement of the drug to a living body as a drug carrier in a drug delivery system. [0003] The application value of temperature sensitivity liposome making an endocyst drug emit in response to a living body's temperature, skin temperature, etc. is [in / medically and pharmacologically / the field of the charge of makeup] high among the above-mentioned stimulus responsibility liposome. Conventionally, as this temperature sensitivity liposome, dimyristoyl phosphatidyl choline, dipalmitoylphosphatidylcholine, JISUTE aroyl phosphatidylcholine, etc. had many things using the phospholipid which has a phase transition point. In phase transition temperature, a lipid changes from the gel state to a liquid crystal condition, turbulence arises in membrane structure and these emit the endocyst matter to it. [0004] Moreover, liposome is covered with the polysaccharide derivative which introduced the Pluronic polymer which has a cloudy point near the room temperature as a part which picks up temperature, and the example which prepared the temperature sensitivity polymer is reported (the collection of the Society of Polymer Science, Japan drafts, 3020 1989 [-3022 or]).

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EFFECT OF THE INVENTION

[Effect of the Invention] As mentioned above, the temperature sensitivity liposome concerning this invention had the very good reactivity over specific temperature. Rather than the phase transition temperature of a liposome configuration lipid, since this good temperature sensitivity becomes settled by the cloudy point which the high molecular compound made to support has, it can also set the temperature to which the endocyst matter is made to emit as desired temperature by adjusting the cloudy point of a high molecular compound.
[0023] Moreover, it was able to be made to emit good in the temperature sensitivity liposome concerning this invention also about the matter which was not able to cause emission sufficient in conventional temperature sensitivity liposome. It is possible to also make emission of a lot of endocyst matter cause for a short time from before by the synergism of change of the membrane structure by the phase transition of a liposome configuration lipid and change of the membrane structure by hydrophobing in the cloudy point of a high molecular compound. Therefore, in conventional temperature sensitivity liposome, when emission of drugs sufficient in a target site is not able to be achieved, there is application value.
[0024] Furthermore, in this invention, since the amount of support in the liposome of a high molecular compound which has a cloudy point is easily controllable, the emission rate of the endocyst matter from temperature sensitivity liposome is also controllable. Therefore, even when it applies to a drug delivery system etc., the difference of an operation of a target site and drugs, effectiveness, etc. can perform a dosage form design with a sustained release drug to the broad pharmaceutical preparation of immediate effect nature.
[0025] Thus, in medicine and the pharmaceutical-sciences field, useful temperature sensitivity liposome was able to be especially offered by this invention.

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] However, in the temperature sensitivity liposome prepared by conventional phospholipid, a setup near temperature was difficult so that the emission temperature of the endocyst matter may serve as a specific value by the lipid which constitutes the liposome film and it may apply in the living body. Moreover, emission of the endocyst matter was also slow and it was what it is hard to use to emit the drug of a considerable amount for a short time. In addition, emission might not take place almost according to the description of the matter which carries out endocyst etc.
[0006] Moreover, the liposome covered with the Pluronic polymer installation polysaccharide derivative was also unsuitable for the target site in the drugs which should be made to act for a short time, although the amount of said polymer introduced on liposome came out only, and did not react for whether being Sumiya to the temperature change for a certain reason but was fit for application to a sustained release drug. Furthermore, control of the amount of installation of the Pluronic polymer to a polysaccharide derivative was also difficult, and it was what cannot perform control of the responsibility or the burst size to a temperature change, and an emission rate easily.

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MEANS

[Means for Solving the Problem] In order to solve the above-mentioned technical problem, when the interior was made to support directly the high molecular compound which has a cloudy point and this high molecular compound carried out hydrophobing at the temperature more than a cloudy point, ****** of liposome changed the membrane structure of liposome, examines the system to which the endocyst matter is made to emit, and came to complete this invention.
[0008] Introduce hydrophobic radicals, such as an alkyl group and cholesterol, into the high molecular compound which is made to carry out the endocyst of the high molecular compound which has a cloudy point to the aqueous phase in liposome, or has a cloudy point, the interaction of this hydrophobic radical and the hydrophobic section of the liposome film is made to produce, and ****** of liposome makes the interior support said high molecular compound in this invention.
[0009] As a high molecular compound which has the cloudy point used by this invention, a polyacrylic acid system polymer can set up a cloudy point near temperature, and is especially desirable. In order to give a cloudy point to a polyacrylic acid system polymer, a straight chain, branched chain, or an annular hydrocarbon group is introduced into the carboxylic acid in a polymer chain, an amide, etc., and a polymer molecule is embellished. For example, the polymerization of the monomers, such as N-pentyl acrylamide, N-isopropyl acrylamide, and N-cyclohexyl acrylamide, can be carried out, and the suitable polymer for the purpose of this invention can be obtained. Furthermore, in case a polyacrylic acid system polymer is prepared, the cloudy point of a high molecular compound can be adjusted by carrying out copolymerization of the monomer from which versatility differs.
[0010] Moreover, if a hydrophobic radical is introduced into a high molecular compound in case especially a liposome front face is made to support said high molecular compound, it can be made to hold to stability. As a hydrophobic radical to introduce, long-chain ****** has a branching alkyl group, desirable cholesterol, etc.
[0011] In case the liposome used by this invention is prepared, there is especially no limit about the lipid to be used. In order to make liposome support a high molecular compound, it adds at the temperature below a cloudy point, the incubation of the solution of a high molecular compound is carried out to a liposome solution for a while, it carries out an interaction to it, or it mixes with a lipid at the time of liposome preparation, and endocyst is carried out into a liposome inland water phase.

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OPERATION

[Function] In the temperature sensitivity liposome which supported the high molecular compound concerning this invention, at the temperature below a cloudy point, if it becomes the temperature more than a cloudy point, the supported high molecular compound serves as hydrophobicity, and although it has spread underwater in the hydrophilic property, it tends to shift into a liposome lipid membrane at the same time a macromolecule chain contracts. Consequently, turbulence arises in the structure of a liposome lipid membrane, and emission of the endocyst matter takes place.
[0013] As described above, in emission of the endocyst matter reacted to the phase transition temperature of a lipid, it may almost be changeless depending on the matter to connote. However, in the case of the temperature sensitivity liposome concerning this invention, when the high molecular compound supported with the phase transition of a lipid serves as hydrophobicity, the burst size of the endocyst matter can be increased remarkably.
[0014] In case the liposome to which the endocyst of the drugs was carried out is applied in the living body, or the liposome to which the endocyst of the active principle was carried out is applied on the skin and the endocyst matter is made to emit in a target site, it is necessary to control an emission rate by an operation of drugs or the target site in various phases. In the temperature sensitivity liposome concerning this invention, the amount of the high molecular compound which liposome is made to support is easily controllable by changing the quantitative ratio of the high molecular compound which carries out an interaction to liposome. Therefore, it is possible to adjust extent of a structural change of the liposome lipid membrane to the temperature more than a cloudy point, and to control the burst size or emission rate of the endocyst matter.

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EXAMPLE

[Example] Furthermore, an example explains this invention to a detail.
[0016] As an example 1, the yolk lecithin multilamellar liposome which made Polly N-isopropyl acrylamide support was prepared. First, copolymerization of N-isopropyl acrylamide and the acrylic-acid stearyl was carried out by the mole ratio of 99:1, the copolymer (average molecular weight 20,000) was compounded, this copolymer water solution and the yolk lecithin multilamellar liposome solution prepared according to the conventional method were incubated at 20 degrees C for 12 hours, and the liposome front face was made to support Polly N-isopropyl acrylamide. Moreover, the carboxy fluorescein was used as endocyst matter.
[0017] About this Polly N-isopropyl acrylamide support liposome, the emission behavior of a carboxy fluorescein with time which carried out endocyst was investigated by measuring fluorescence intensity. The yolk lecithin liposome which is not making Polly N-isopropyl acrylamide support was made into the example 1 of a comparison at that time. A result is shown in drawing 1 .
[0018] In drawing 1 , the liposome of an example 1 showed the emission behavior of a carboxy fluorescein comparable as the liposome of the example 1 of a comparison at 20 degrees C, and the emission rate was controlled considerably. However, when temperature was raised at 34 degrees C, emission of a remarkable carboxy fluorescein was accepted in the liposome of an example 1. This emission was controlled when temperature was again reduced at 20 degrees C. On the other hand, the remarkable reaction to such temperature was not accepted in the liposome of the example 1 of a comparison. The remarkable temperature sensitivity in an example 1 is considered that the Polly N-isopropyl acrylamide which this liposome was made to support is because it has a cloudy point at 31 degrees C.
[0019] Next, the dipalmitoylphosphatidylcholine uni-lamellae liposome which made Polly N-isopropyl acrylamide support was prepared as an example 2. First, copolymerization of N-isopropyl acrylamide and the acrylic-acid stearyl was carried out by the mole ratio of 99:1, the copolymer (mean molecular weight 20,000) was compounded, this copolymer water solution and the dipalmitoylphosphatidylcholine uni-lamellae liposome solution prepared according to the conventional method were incubated at 20 degrees C for 12 hours, and the liposome front face was made to support Polly N-isopropyl acrylamide. Moreover, calcein was used as endocyst matter.
[0020] About this Polly N-isopropyl acrylamide support liposome, change by the temperature of the emission behavior of calcein which carried out endocyst was investigated by measuring fluorescence intensity. The dipalmitoylphosphatidylcholine liposome to which Polly N-isopropyl acrylamide is not carried out at the time of ** was made into the example 2 of a comparison at that time. The rate (leakage rate) to the intensive matter of the burst size of the calcein 1 minute [in each temperature] after measurement initiation showed the result to drawing 2 .
[0021] When the endocyst of the calcein is carried out, in usual lipid liposome, most change of emission by the phase transition temperature of a lipid is not accepted. Also in drawing 2 , although the phase transition temperature of dipalmitoylphosphatidylcholine was 42 degrees C, by the liposome of the example 2 of a comparison, the leakage of calcein was only accepted more slightly [in an elevated-temperature side] than near 42 degree C. On the other hand, in

the liposome of an example 2, emission of calcein was promoted from near 31 degree C which is the cloudy point of Polly N-isopropyl acrylamide, and remarkable emission was further accepted near 42 degree C for a short time. In the liposome of an example 2, since the phase transition temperature of the dipalmitoylphosphatidylcholine from which the cloudy point of Polly N-isopropyl acrylamide constitutes 31 degrees C and liposome is 42 degrees C, it is thought that increase of emission of the endocyst matter is accepted in the large temperature requirement near [near 30 degree C to] 42 degree C. Moreover, even endocyst matter which is not usually emitted was able to make the considerable amount emit.

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is drawing showing the emission behavior of the carboxy fluorescein from liposome in 20 degrees C and 34 degrees C with time about the example 1 and the example 1 of a comparison of this invention.
[Drawing 2] It is drawing showing the leakage rate of the calcein from liposome, and relation with temperature about the example 2 and the example 2 of a comparison of this invention.
[Description of Notations]
1 Example 1
2 Example 1 of Comparison
3 Example 2
4 Example 2 of Comparison

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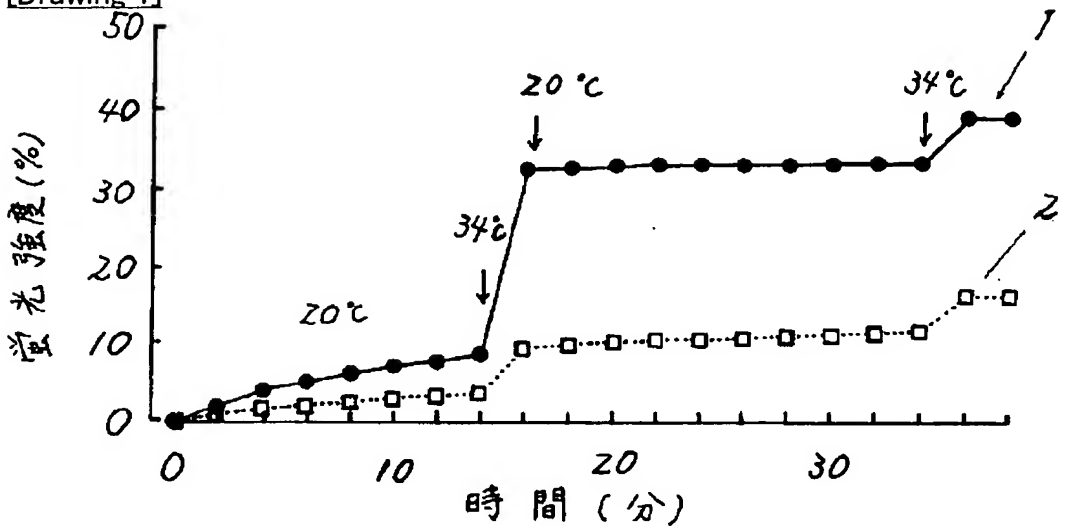
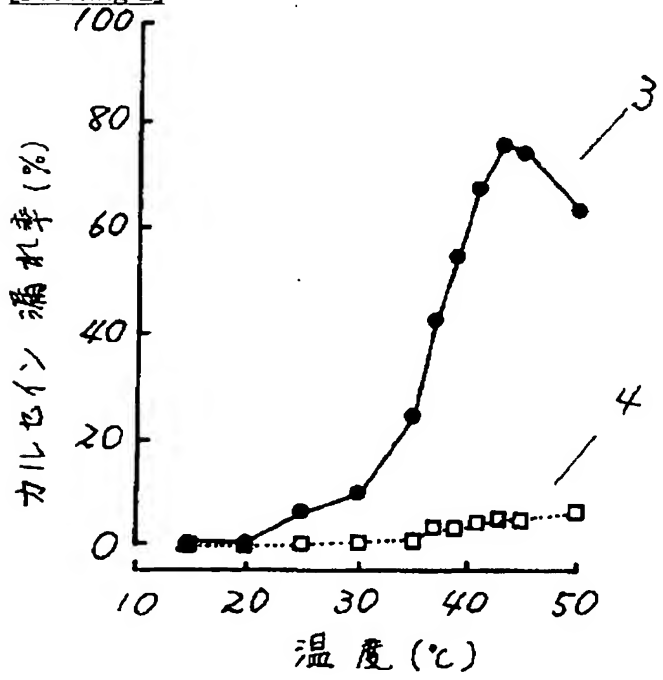
DRAWINGS

[Drawing 1]



[Drawing 2]



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特開平5－228358

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Ｂ０１Ｊ　13/02				
Ａ６１Ｋ　9/127		Ｒ　7329－4Ｃ		
		Ｆ　7329－4Ｃ		
47/32		Ｃ　7433－4Ｃ		
		8317－4Ｇ	Ｂ０１Ｊ　13/02　Ｚ	

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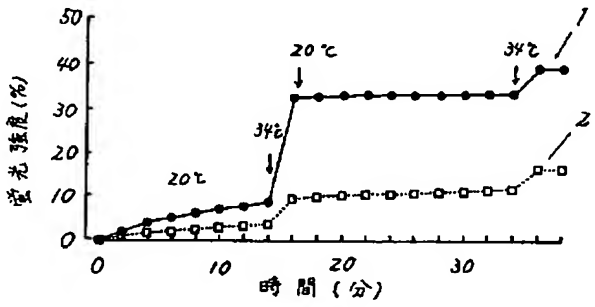
(54)【発明の名称】　温度感受性リポソーム

(57)【要約】

【目的】　特定の温度において内包物質を放出することができ、ドラッグデリバリーシステムにおける薬物担体等、医学・薬学分野において応用可能な温度感受性リポソームを得る。

【構成】　従来の脂質リポソームの表面又は内部に、曇点を有する高分子化合物を担持させ、温度感受性リポソームを得る。このリポソームにおいては、曇点付近の温度でリポソーム表面又は内部に担持させた高分子化合物が疎水化するため、リポソーム膜の構造に乱れが生じ、内包物質の放出が起こる。曇点を有する高分子化合物としては、ポリ-N-イソプロピルアクリルアミド等のポリアクリル酸系ポリマーが好適である。また、アルキル基，コレステロール等の疎水性基を導入すると、リポソーム表面に安定に担持させることができる。



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【特許請求の範囲】
【請求項１】　曇点を有する高分子化合物を表面又は内部に担持させて成る、温度感受性リポソーム。
【請求項２】　曇点を有する高分子化合物が、ポリアクリル酸系ポリマーであることを特徴とする、請求項１に記載の温度感受性リポソーム。
【請求項３】　曇点を有する高分子化合物に、疎水性基を導入して表面又は内部に担持させて成る、温度感受性リポソーム。
【請求項４】　曇点を有する高分子化合物が、ポリアクリル酸系ポリマーであることを特徴とする、請求項３に記載の温度感受性リポソーム。
【発明の詳細な説明】
【０００１】
【産業上の利用分野】本発明は、医薬品，化粧料をはじめ、医学・薬学分野で応用し得る、温度変化に対応して内包物質を放出し得る温度感受性リポソームに関する。
【０００２】
【従来の技術】リポソームは、医学・薬学分野において、薬物などの担体や細胞膜モデル等として幅広く応用されている。最近では、リポソームを高分子化して膜の強化を図ったり、糖や酵素，タンパク質等を固定化して、刺激応答性リポソームを調製し、生体への薬物の吸収性改善の目的で、ドラッグデリバリーシステムにおける薬物運搬体として利用が検討されたりしている。
【０００３】上記の刺激応答性リポソームのうち、温度感受性リポソームは、生体の体温や皮膚温等に反応して内包薬物を放出させるなど、医学的・薬学的にも、或いは化粧料の分野においても、応用価値の高いものである。従来、かかる温度感受性リポソームとしては、ジミリストイルホスファチジルコリン，ジパルミトイルホスファチジルコリン，ジステアロイルホスファチジルコリン等、相転移点を有するリン脂質を用いたものが多かった。これらは、相転移温度において脂質がゲル状態から液晶状態に変化し、膜構造に乱れが生じて内包物質を放出するものである。
【０００４】また、温度を感受する部位として、室温付近に曇点を有するプルロニックポリマーを導入した多糖誘導体でリポソームを被覆し、温度感受性ポリマーを調製した例が報告されている（高分子学会予稿集，３０２０－３０２２，１９８９年）。
【０００５】
【発明が解決しようとする課題】しかし、従来のリン脂質により調製した温度感受性リポソームにおいては、内包物質の放出温度はリポソーム膜を構成する脂質により特定の値となり、生体内に適用するべく体温付近での設定は困難であった。また、内包物質の放出も遅く、短時間に相当量の薬物を放出させたい場合には使用しにくいものであった。その他に、内包させる物質の性状等によっては、ほとんど放出の起こらないこともあった。

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【０００６】また、プルロニックポリマー導入多糖誘導体で被覆したリポソームも、リポソーム上に導入される前記ポリマーの量がわずかであるため、温度変化に対しすみやかに反応せず、徐放性製剤への応用には向くが、標的部位に短時間で作用させるべき薬剤等においては不向きであった。さらに、多糖誘導体へのプルロニックポリマーの導入量の制御も困難で、温度変化に対する応答性や放出量及び放出速度の制御を行い難いものであった。
【０００７】
【課題を解決するための手段】上記の課題を解決するため、リポソームの表面又は内部に、曇点を有する高分子化合物を直接担持させ、曇点以上の温度でこの高分子化合物が疎水化することにより、リポソームの膜構造を変化させ、内包物質を放出させる系を検討し、本発明を完成させるに至った。
【０００８】本発明においては、曇点を有する高分子化合物をリポソーム中の水相に内包させるか、曇点を有する高分子化合物にアルキル基，コレステロール等の疎水性基を導入し、この疎水性基とリポソーム膜の疎水性部との相互作用を生ぜしめて、前記高分子化合物をリポソームの表面又は内部に担持させる。
【０００９】本発明で使用する曇点を有する高分子化合物としては、ポリアクリル酸系ポリマーが体温付近に曇点を設定でき、特に好ましい。ポリアクリル酸系ポリマーに曇点を持たせるには、ポリマー鎖中のカルボン酸，アミド等に、直鎖，分岐鎖或いは環状の炭化水素基等を導入して、ポリマー分子の修飾を行う。たとえば、N-ペンチルアクリルアミド，N-イソプロピルアクリルアミド，N-シクロヘキシルアクリルアミド等のモノマーを重合させて、本発明の目的に好適なポリマーを得ることができる。さらに、ポリアクリル酸系ポリマーを調製する際、種々の異なるモノマーを共重合させることにより、高分子化合物の曇点の調節を行うことができる。
【００１０】また、特にリポソーム表面に前記高分子化合物を担持させる際、高分子化合物に疎水性基を導入すると、安定に保持させることができる。導入する疎水性基としては、長鎖の直鎖又は分岐アルキル基，コレステロール等が好ましい。
【００１１】本発明で用いるリポソームを調製する際、使用する脂質については特に制限はない。リポソームに高分子化合物を担持させるには、リポソーム溶液に高分子化合物の溶液を曇点以下の温度で添加し、しばらくインキュベーションして相互作用させるか、或いはリポソーム調製時に脂質と混合し、リポソーム内水相中に内包させる。
【００１２】
【作用】本発明に係る高分子化合物を担持した温度感受性リポソームにおいて、曇点以下の温度では、担持された高分子化合物は親水性で水中に広がっているが、曇点

以上の温度になると、疎水性となり、高分子鎖が収縮すると同時にリポソーム脂質膜内へ移行しようとする。その結果、リポソーム脂質膜の構造に乱れが生じ、内包物質の放出が起こる。

【００１３】前記したように、脂質の相転移温度に反応した内包物質の放出においては、内包する物質によってはほとんど変化がない場合がある。しかし、本発明に係る温度感受性リポソームの場合、脂質の相転移とともに担持された高分子化合物が疎水性となることにより、内包物質の放出量を著しく増大させることができる。

【００１４】薬剤を内包させたリポソームを生体内に適用したり、有効成分を内包させたリポソームを皮膚上に適用し、内包物質を標的部位で放出させる際、薬剤の作用や標的部位により、放出速度を種々の段階に制御する必要がある。本発明に係る温度感受性リポソームにおいては、リポソームと相互作用させる高分子化合物の量比を変化させることにより、リポソームに担持させる高分子化合物の量を容易に制御できる。従って、曇点以上の温度に対するリポソーム脂質膜の構造変化の程度を調節して、内包物質の放出量或いは放出速度を制御することが可能である。

【００１５】

【実施例】さらに、本発明について実施例により詳細に説明する。

【００１６】実施例１として、ポリ-N-イソプロピルアクリルアミドを担持させた卵黄レシチンマルチラメラリポソームを調製した。まず、N-イソプロピルアクリルアミドとアクリル酸ステアリルとを９９：１のモル比で共重合させて共重合体（平均分子量２０，０００）を合成し、この共重合体水溶液と、常法に従い調製した卵黄レシチンマルチラメラリポソーム溶液とを２０℃で１２時間インキュベートして、リポソーム表面にポリ-N-イソプロピルアクリルアミドを担持させた。また、内包物質として、カルボキシフルオレセインを用いた。

【００１７】このポリ-N-イソプロピルアクリルアミド担持リポソームについて、内包させたカルボキシフルオレセインの経時的な放出挙動を、蛍光強度を測定することにより調べた。その際、ポリ-N-イソプロピルアクリルアミドを担持させていない卵黄レシチンリポソームを比較例１とした。結果を図１に示す。

【００１８】図１において、実施例１のリポソームは、２０℃では比較例１のリポソームと同程度のカルボキシフルオレセインの放出挙動を示し、放出速度はかなり抑制されていた。しかし、温度を３４℃に上昇させると、実施例１のリポソームにおいては、顕著なカルボキシフルオレセインの放出が認められた。温度を再び２０℃に低下させると、この放出は抑制された。一方、比較例１のリポソームにおいては、このような温度に対する顕著な反応は認められなかった。実施例１における顕著な温度感受性は、このリポソームに担持させたポリ-N-イソプロピルアクリルアミドが、３１℃に曇点を有するためであると考えられる。

【００１９】次に、実施例２として、ポリ-N-イソプロピルアクリルアミドを担持させた、ジパルミトイルホスファチジルコリンユニラメラリポソームを調製した。まず、N-イソプロピルアクリルアミドとアクリル酸ステアリルとを９９：１のモル比で共重合させて共重合体（平均分子量２０，０００）を合成し、この共重合体水溶液と、常法に従い調製したジパルミトイルホスファチジルコリンユニラメラリポソーム溶液とを２０℃で１２時間インキュベートして、リポソーム表面にポリ-N-イソプロピルアクリルアミドを担持させた。また、内包物質として、カルセインを用いた。

【００２０】このポリ-N-イソプロピルアクリルアミド担持リポソームについて、内包させたカルセインの放出挙動の温度による変化を、蛍光強度を測定することにより調べた。その際、ポリ-N-イソプロピルアクリルアミドを担時させていないジパルミトイルホスファチジルコリンリポソームを比較例２とした。結果は、各温度における測定開始１分後のカルセインの放出量の内包量に対する割合（漏れ率）にて、図２に示した。

【００２１】カルセインを内包させた場合、通常の脂質リポソームでは、脂質の相転移温度による放出の変化はほとんど認められない。図２においても、ジパルミトイルホスファチジルコリンの相転移温度は４２℃であるが、比較例２のリポソームでは、４２℃付近より高温側でわずかにカルセインの漏れが認められただけであった。一方、実施例２のリポソームでは、ポリ-N-イソプロピルアクリルアミドの曇点である３１℃付近からカルセインの放出が促進され、さらに、４２℃付近では短時間で著しい放出が認められた。実施例２のリポソームにおいては、ポリ-N-イソプロピルアクリルアミドの曇点が３１℃、リポソームを構成するジパルミトイルホスファチジルコリンの相転移温度が４２℃であるため、３０℃付近から４２℃付近の広い温度範囲で内包物質の放出の増大が認められると考えられる。また、通常は放出されないような内包物質でさえも、相当量の放出を行わせることができた。

【００２２】

【発明の効果】以上のように、本発明に係る温度感受性リポソームは、特定の温度に対する反応性が非常に良いものであった。この良好な温度感受性は、リポソーム構成脂質の相転移温度よりも、担持させた高分子化合物の有する曇点により定まるため、高分子化合物の曇点を調節することによって、内包物質を放出させる温度を所望の温度に設定することも可能である。

【００２３】また、本発明に係る温度感受性リポソームにおいては、従来の温度感受性リポソームでは十分な放出を起こすことのできなかった物質についても、良好に放出させることができた。リポソーム構成脂質の相転移

による膜構造の変化と、高分子化合物の曇点における疎水化による膜構造の変化との相乗作用により、従来より短時間に多量の内包物質の放出を起こさせることも可能である。従って、従来の温度感受性リポソームでは標的部位で十分な薬剤の放出を果たせなかった場合などにも、応用価値があるものである。

【００２４】さらに、本発明においては、曇点を有する高分子化合物のリポソームにおける担持量を容易に制御することができるため、温度感受性リポソームからの内包物質の放出速度をも制御することができる。従って、ドラッグデリバリーシステム等に応用した場合でも、標的部位や薬剤の作用，効果等の相違により、徐放性製剤から即効性の製剤まで、幅広い製剤設計を行うことができる。

【００２５】このように、本発明により、医学・薬学分野において特に有用な温度感受性リポソームを提供することができた。

【図面の簡単な説明】

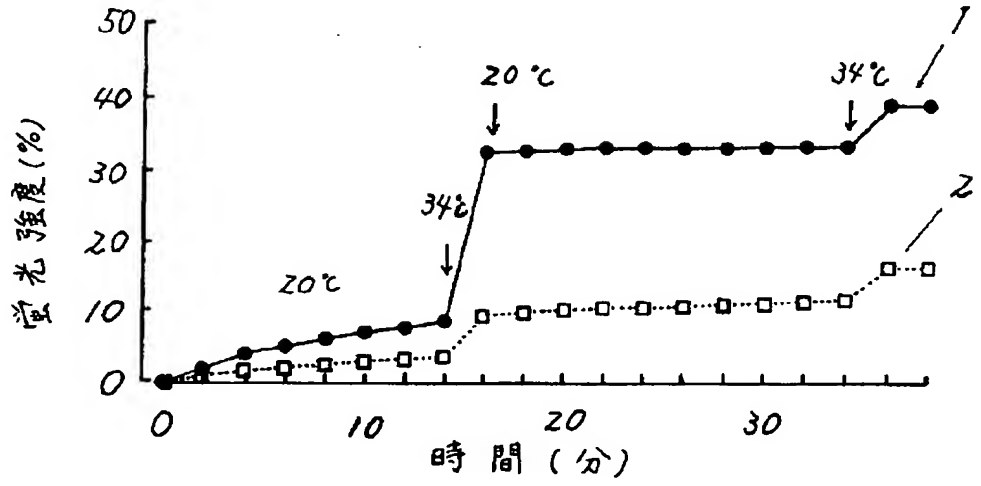
【図１】本発明の実施例１及び比較例１について、２０℃及び３４℃における、リポソームからのカルボキシフルオレセインの放出挙動を経時的に示す図である。

【図２】本発明の実施例２及び比較例２について、リポソームからのカルセインの漏れ率と、温度との関係を示す図である。

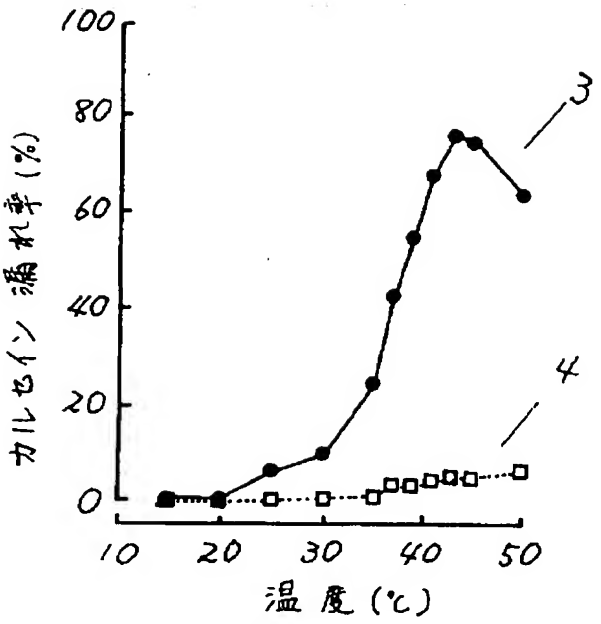
【符号の説明】

１　実施例１
２　比較例１
３　実施例２
４　比較例２

【図１】



【図２】



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